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Insecticidal effect of volatile compounds from plant materials of *Murraya exotica* against Red Imported Fire Ant Workers

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Abstract
The effect of volatile compounds from the mashed fresh, fallen, and dried leaves of *Murraya exotica* on the behavior of red imported fire ant (*Solenopsis invicta*, RIFA) workers was investigated by fumigation toxicity bioassay. The volatile compounds from different mashed leaves (fresh, fallen, and dried leaves) of *M. exotica* were collected by solid-phase microextraction and identified by gas chromatography–mass spectrometry. β-caryophyllene, α-cedrene, α-copaene, β-cubebene, and germacrene D were identified as major components of the volatile compounds. In exposure time from 1 d to 9 d, the mortality of RIFA increased from 5.00% to 100.00% (fresh leaves), 11.67% to 93.33% (fallen leaves), and 15.00% to 83.33% (dried leaves) in minor workers, whereas in major workers, the increases were from 13.33% to 93.33% (fresh leaves), 6.67% to 83.33% (fallen leaves), and 10.00% to 60.00% (dried leaves). The volatile compounds reduced the walking and grasping abilities and aggregation rate of RIFA workers. Results indicate that mashed leaves of *M. exotica* have potential for controlling RIFA.

Introduction

Red imported fire ant (*Solenopsis invicta* Buren 1972 (Myrmicinae), RIFA), native from Parana River basin of South America, is a voracious consumer of numerous dead animals, such as arthropods, earthworms and vertebrates (Nattrass & Vanderwoude, 2001). RIFAs have become a pest in southern United States, Australia, New Zealand, Thailand, Taiwan, and the Philippines, and they were introduced to southern Chinese Provinces of Guangdong, Guangxi, and Fujian in 2005 (Ascunce et al., 2011; Zhang et al., 2007). In several highly infested areas, RIFAs have caused the decline of native ant species by up to 90% through displacement (Porter & Savignano, 1990). RIFAs have caused severe damage to human, animals, agriculture, and environment. Furthermore, these ants negatively affect the local biodiversity and cause approximately US $5 billion losses yearly in urban and agricultural areas in the United States (Cheng et al., 2008). Traditional methods such as insecticides and baits are used for managing *S. invicta*; however, these methods cause pollution to the environment. Therefore, new methods such as natural and environment-friendly insecticides should be found to control RIFAs (Vogt et al., 2002; Appel et al., 2004).

Murraya exotica belongs to the family Rutaceae and is an evergreen shrub or occasionally a small tree, which is mainly distributed in the west and southeast of China. Its flowers are few, white and fragrant (Zhang et al., 2008). Previous studies have reported the chemical compositions of essential oils from flowers, leaves, and stems of *M. exotica* (Pino et al., 2006; Raina et al., 2006; Rout et al., 2007; Olawore et al.,

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2005; Negi et al., 2005). Yu et al. (2001) reported that *M. exotica* extract on some fungi has significant inhibitory effect. Li et al. (2001) found the essential oils of *M. exotica* have good control effect on stored grain pests. Moreover, Luo et al. (2005) found that the extracts of *M. exotica* indicated very strong antifeedant activities against the third instar and fifth instar larvae. However, no research has yet been conducted on the insecticidal activity of the essential oil of *M. exotica* against RIFAs. This study is the first to report on the toxicity of the volatile compounds released from the mashed leaves of *M. exotica* against RIFAs. Moreover, the present study investigates the effects of the volatile compounds from the mashed leaves of *M. exotica* on the RIFA workers.

Materials and methods

Plant materials

The leaves of *M. exotica* were collected from the Insecticidal Botanical Garden at the South China Agricultural University. Three different leaves of *M. exotica* were used for the test. The fresh leaves were cut off and immediately sent to the laboratory. The fallen leaves were collected from the ground below *M. exotica*. The dried leaves were gained by putting the fresh leaves in baking box with 40 °C for 5 h.

Insects

S. invicta colonies were obtained from a suburb in Guangzhou and stored in the laboratory for bioassays in plastic containers at 25 ± 2 °C and 60% to 80% RH. A test tube (25 mm × 200 mm) partially filled with 10% honey water and plugged with cotton was used as a water source, and a Petri dish (8.5 cm × 1.5 cm) containing the larvae of *Tenebrio molitor* L. (Coleoptera Tenebrionidae) was used as a food source. RIFAs were kept in a dry indoor environment at 25 ± 2 °C until the experiment was over.

Fumigation toxicity bioassay

Fresh, fallen, and dried leaves of *M. exotica* were crushed thoroughly in a blender for 5 min. About 30 g of mashed leaves was placed on the bottom of a 1000 ml breaker. Twenty minor workers (body length = 2.6 mm to 3.0 mm, head width = 0.5 mm to 0.6 mm) and 10 major workers (body length = 4.2 mm to 4.6 mm, head width = 1.0 mm to 1.2 mm) were placed on the bottom of a 100 ml breaker. The breaker was coated with Fluon emulsion inside vertical wall to prevent the ants from escaping and placed on the bottom of a 1000 ml breaker without covering the mashed leaves. The 1000 ml beaker was covered with plastic film. The ants were placed on the laboratory maintained at 25 ± 2 °C and 65% ± 5% relative humidity. All treatments were replicated thrice. The contrast was the absence of mashed leaves in the 1000 ml beaker.

Physiological index observation of RIFA workers

The mortalities of the workers were observed 1, 3, 5, 7, and 9 d after adding the 100 ml beaker to the leaves. Behavioral observation on the grasping ability of the workers was determined 1, 3, 5, 7, and 9 d after adding the 100 ml beaker to the leaves. The workers were placed on an A4 paper (made from plant fibre, 210 mm × 297 mm), which was slowly turned over to 180 degrees for 1 min. If the ants would not fall down from the A4 paper, they were regarded as possessing grasping ability.

The formula was as follows: grasping rate = number of worker ants possessing grasping ability/number of worker ants per replicate × 100.

Behavioral observation on the walking ability of the workers was determined 1, 3, 5, 7, and 9 d after testing. The workers were placed on an A4 paper. The ants were regarded as possessing walking ability if they could walk continuously for 10 cm without falling down from the A4 paper.

The formula was as follows: walking rate = number of worker ants possessing walking ability/number of worker ants per replicate × 100.

Behavioral observation on the aggregation of the workers was determined 1, 3, 5, 7, and 9 d after testing. The aggregating level based on the method employed by Depickere et al. (2004) and Devigne et al. (2011). The workers were regarded as aggregating if over two workers gathered, and the distance between each other was less than 0.5 cm.

The formula was as follows: aggregation rate = number of worker ants in aggregate mass/number of worker ants per replicate × 100.

Extraction of volatiles by solid-phase microextraction

Fresh, fallen, and dried leaves were mashed with a high-speed organization stamp mill for 5 min, and then 30 g of mashed leaves was placed into a 250 ml glass Erlenmeyer flask covered with silver paper.

The manual solid-phase microextraction device equipped with a 100 μm polydimethylsiloxane fiber (Supelco) was employed in this study. The fiber was activated at constant temperature (250 °C) for 30 min before use. The activated fiber was then pushed into the Erlenmeyer flask contained mashed leaves to absorb the volatiles for 40 min. Fiber was then poured into the injection port of the gas chromatography–mass spectrometry (GC–MS) with a temperature of 250 °C for 3 min, and the volatiles were analyzed by GC–MS.

Chemical Analysis by Gas Chromatography–Mass spectrometry

The sample was detected by an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometer detector. A DB-5 capillary column (30.00 m × 0.25 mm; film thickness by 0.25 mm) was held at 50 °C for 1 min, raised to 200 °C at the rate of 3°C/min for 2 min, and raised to 230 °C (10 °C/min) for 2 min. The injection temperature was set at 230

°C. The detector was operated at 280 °C. Helium was used as a carrier gas at a flow rate of 1 ml/min. The compounds were identified by retention times, Kovats indices, and mass spectra.

Statistical Analysis

Data were transformed into arcsine square root values for a three-way analysis of variance (ANOVA) to determine the significance of the effects of plant material, exposure time, mortalities, grasping rate, walking rate, and aggregation of the minor and major workers as well as various interactions. Furthermore, the differences in the data were assessed using Duncan's multiple range test, with $P < 0.05$ considered statistically significant. The figures were generated using Microsoft Office Excel 2007.

Results

Results of ANOVA

The mortalities, grasping rate, walking rate, and aggregation rate of workers may vary significantly according to plant material, exposure time, and worker size (ANOVA, $P < 0.05$). The results reveal that the three main effects and the partial interactions are significant (Table 1). However, the mortalities, grasping rate, walking rate, and aggregation rate of RIFAs had no difference between with the interaction exposure time × worker size (P=0.2626, 0.2544, 0.2431, 0.3612) and the interaction plant material × exposure time × worker size (P=0.1496, 0.4848, 0.4598, 0.0922).

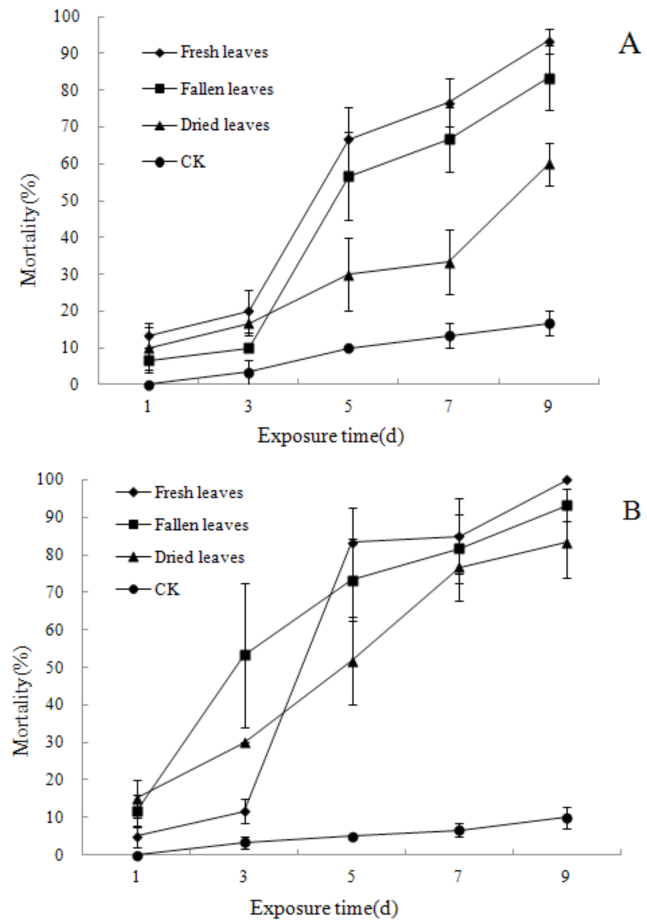

Fig 1. Mortalities of workers (A: major workers, B: minor workers) after treated with different mashed leaves of *M. exotica.*

Table 1. ANOVA for the main factors of the fumigation test affecting the behaviors of RIFA.

Factors	df	Mortality		Grasping rate		Walking rate		Aggregation rate	
		F values	P values	F values	P values	F values	P values	F values	P values
A	3	105.002	0.0001	105.558	0.0001	107.198	0.0001	14.0164	0.0001
B	4	104.588	0.0001	109.724	0.0001	113.959	0.0001	47.0154	0.0001
C	1	19.3056	0.0001	16.8368	0.0001	17.2425	0.0001	43.0126	0.0001
A×B	12	9.9821	0.0001	10.3586	0.0001	10.7487	0.0001	4.4143	0.0001
A×C	3	7.3649	0.0002	6.3701	0.0006	6.9493	0.0003	0.3572	0.784
B×C	4	1.3393	0.2626	1.3625	0.2544	1.3953	0.2431	1.1025	0.3612
A×B×C	12	1.479	0.1496	0.9694	0.4848	0.9967	0.4598	1.6587	0.0922

A: Plant material, B: Exposure time, C: Worker size (P=0.05)

Fumigation toxicity bioassay

According to Fig 1, both major and minor workers exposed to volatiles from mashed leaves of *M. exotica* show increased mortality over time.

After 1 d exposure, the volatile compounds of fresh, fallen, and dried leaves of *M. exotica* lead to 5%, 11.6%, and 10% mortality in minor workers as well as 13.33%, 6.67%, and 15.00% mortality in major workers. However, the mortality rates are 100.00%, 93.33%, and 83.33% for the minors as well as 93.33%, 83.33%, and 83.33% for the majors at 9 d of treatment.

Grasping, walking, and aggregation rate

Compared with the mortality of fumigation toxicity, the grasping, walking, and aggregation rates of RIFAs caused by volatiles from mashed leaves of *M. exotica* decrease over time (Figs 2, 3, and 4). At exposure times ranging from 1 d to 9 d, the grasping abilities decrease from 86.7% to 6.7% (fresh leaves), 93.3% to 16.7% (fallen leaves), and 96.7% to 40.0% (dried leaves) for the major workers and from 95.0% to 0.0% (fresh leaves), 88.3% to 6.7% (fallen leaves), and 85.0% to 16.7% (dried leaves) for the minor workers (Fig 2,Table 3); the walking abilities are reduced from 86.7% to 6.7% (fresh leaves), 93.3% to 16.7% (fallen leaves), and 90.0% to 40.0% (dried leaves) for the major workers, as well as from 95.0% to 0.0% (fresh leaves), 88.3% to 6.7% (fallen leaves), and 85.0% to 16.7% (dried leaves) for the minor workers (Fig 3, Table 4); the aggregation rate is reduced from 50.0% to 0.0% (fresh leaves), 86.7% to 16.7% (fallen leaves), and 50.0% to 40.0% (dried leaves) for the major workers and from 31.7% to 0.0% (fresh leaves), 68.3% to 3.3% (fallen leaves), and 51.7% to 13.3% (dried leaves) for the minor workers (Fig 4, Table5).

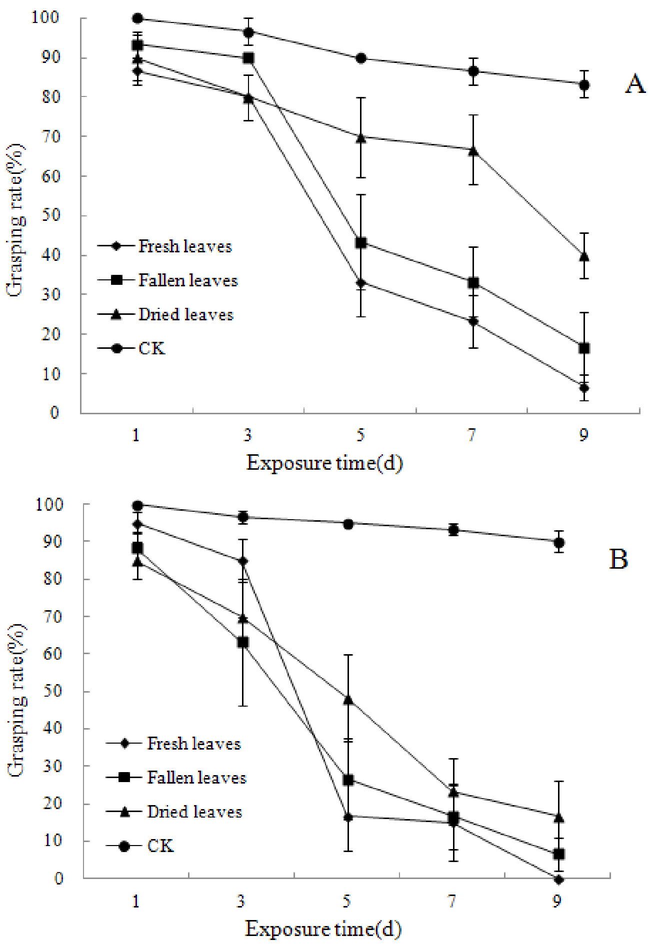

Fig 2. Effects on grasping ability of workers (A:major workers, B: Minor workers) after treated with different mashed leaves of *M. exotica.*

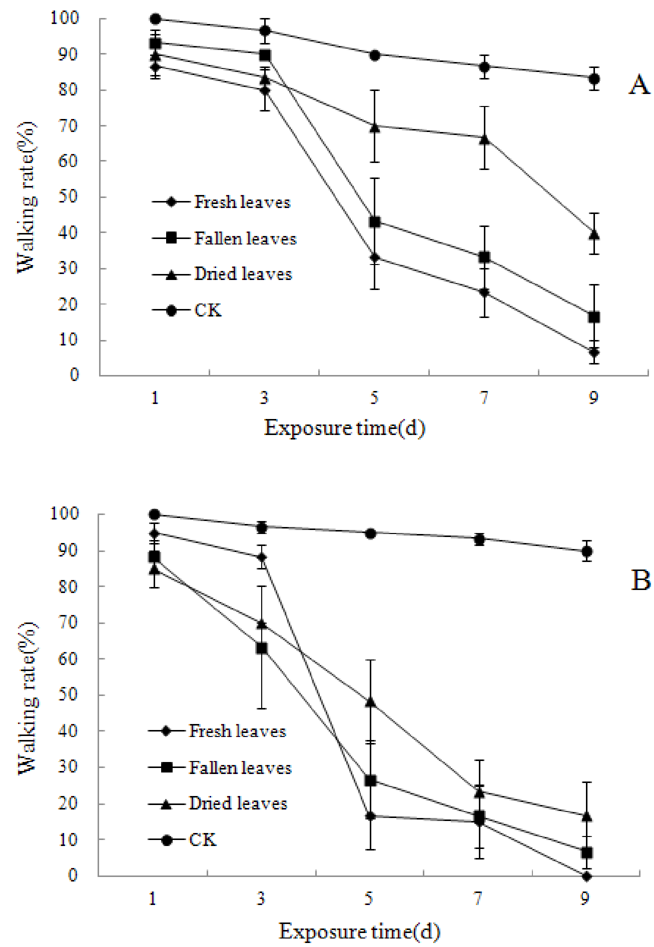

Fig 3. Effects on walking ability of workers (A: major workers, B: minor workers) after treated with different mashed leaves of *M. exotica.*

Table 2. Mortality of workers caused by mashed fresh, fallen, and dried leaves of *M. exotica* in the fumigation bioassay.

Leaves	Workers	Mortality (%, mean±SE)				
		1d	3d	5d	7d	9d
Fresh leaves	major	13.3±3.3	20.0±5.8	66.7±8.8	76.7±6.7	93.3±3.3
	minor	5.0±2.9	11.7±3.3	83.3±9.3	85.0±10.0	100.0±0.0
Fallen leaves	major	6.7±3.3	10.0±0.0	56.7±12.0	66.7±8.8	83.3±8.8
	minor	11.7±4.4	53.3±19.2	73.3±10.9	81.7±9.3	93.3±4.4
Dried leaves	major	10.0±3.3	16.7±3.3	30.0±10.0	33.3±8.8	60.0±5.8
	minor	15.0±5.0	30.0±0.0	51.7±11.7	76.7±8.8	83.3±9.3
ck	major	0.0±0.0	3.3±3.3	10.0±0.0	13.3±3.3	16.7±3.3
	minor	0.0±0.0	3.3±1.7	5.0±0.0	6.7±2.9	10.0±2.9

Table 3. Effects on grasping rate of workers after treated with mashed fresh, fallen, and dried leaves of *M. exotica.*

Leaves	Workers	Grasping rate (%, mean±SE)				
		1d	3d	5d	7d	9d
Fresh	major	86.7±3.3	80.0±5.8	33.3±8.8	23.3±6.7	6.7±3.3
leaves	minor	95.0±2.9	85.0±5.8	16.7±9.3	15.0±10.0	0.0±0.0
Fallen	major	93.3±3.3	90.0±0.0	43.3±12.0	33.3±8.8	16.7±8.8
leaves	minor	88.3±4.4	63.3±16.9	26.7±10.9	16.7±8.8	6.7±4.4
Dried	major	90.0±5.8	80.0±5.8	70.0±10.0	66.7±8.8	40.0±5.8
leaves	minor	85.0±5.0	70.0±0.0	48.3±11.7	23.3±8.8	16.7±9.3
ck	major	100.0±0.0	96.7±3.3	90.0±0.0	86.7±3.3	83.3±3.3
	minor	100.0±0.0	96.7±1.7	95.0±0.0	93.3±1.7	90.0±2.9

Table 4. Effects on walking rate of workers after treated with mashed fresh, fallen, and dried leaves of *M. exotica.*

Leaves	Workers	Walking rate (%, mean±SE)				
		1d	3d	5d	7d	9d
Fresh	major	86.7±3.3	80.0±5.8	33.3±8.8	23.3±6.7	6.7±3.3
leaves	minor	95.0±2.9	88.3±3.3	16.7±9.3	15.0±10.0	0.0±0.0
Fallen	major	93.3±3.3	90.0±0.0	43.3±12.0	33.3±8.8	16.7±8.8
leaves	minor	88.3±4.4	63.3±16.9	26.7±10.9	16.7±8.8	6.7±4.4
Dried	major	90.0±5.8	80.0±3.3	70.0±10.0	66.7±8.8	40.0±5.8
leaves	minor	85.0±5.0	70.0±0.0	48.3±11.7	23.3±8.8	16.7±9.3
ck	major	100.0±0.0	96.7±3.3	90.0±0.0	86.7±3.3	83.3±3.3
	minor	100.0±0.0	96.7±1.7	95.0±0.0	93.3±1.7	90.0±2.9

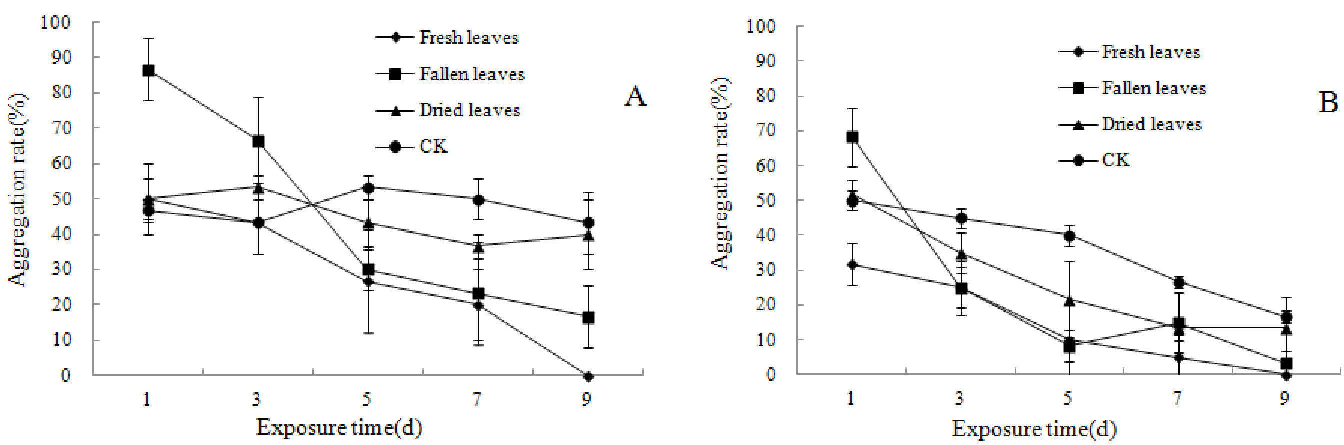

Fig. 4 Effects on aggregation of workers (A: major workers, B: minor workers) after treated with different mashed leaves of *M. exotica.*

Chemical compositions of mashed leaves of ***Murraya exotica***

Figure 5 shows the GC–MS total ion chromatograms of extracts of fresh, fallen, and dried leaves of *M. exotica*. The result demonstrates that the volatile compounds from the mashed fresh, fallen, and dried of *M. exotica* contain 20, 28, and 21 major constituents, respectively (Tables 6, 7, and 8). The major components of fresh leaves comprising 89.82% of the total volatile compound are identified as β-caryophyllene (40.20%), (+)-β-himachalene (17.76%), linalool (8.81%), 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl, [S-(R*,S*)]-(7.00%), germacrene D (6.28%), (E)-β-farnesene (3.67%), a-copaene (3.09%), and humulene (3.01%). For fallen leaves, the 67.31% major components of the total volatile include β-caryophyllene (25.30%), α-cedrene (14.01%), curcumene (10.56%), trans-α-bergamotene (9.06%), germacrene D (6.90%), (1R)-(+)-α-pinene (5.00%), and α-copaene (3.38%). The major components of dried leaves comprising 77.14% of the total volatile compound are α-cedrene (25.05%), β-caryophyllene (23.43%), germacrene D (9.13%), β-Cubebene (8.79%), benzene, 1-(1,5-dimethylhexyl)-4-methyl-(7.56%), trans-α-bergamotene (6.38%), and α-copaene (3.18%).

Table 5. Effects on aggregation rate of workers after treated with mashed fresh, fallen, and dried leaves of *M. exotica.*

Leaves	Workers	Aggregation rate (%, mean±SE)				
		1d	3d	5d	7d	9d
Fresh leaves	major	50.0±5.8	43.3±8.8	26.7±14.5	20.0±10.0	0.0±0.0
	minor	31.7±6.0	25.0±5.8	10.0±10.0	5.0±5.0	0.0±0.0
Fallen leaves	major	86.7±8.8	66.7±12.0	30.0±5.8	23.3±14.5	16.7±8.8
	minor	68.3±8.3	25. 0±7.6	8.3±4.4	15.0±8.7	3.3±3.3
Dried leaves	major	50.0±10.0	53.3±3.3	43.3±6.7	36.7±3.3	40.0±10.0
	minor	51.7±4.4	35.0±5.8	21.7±10.9	13.3±1.7	13.3±8.8
ck	major	46. 7±3.3	43.3±8.8	53.3±3.3	50.0±5.8	43.3±8.8
	minor	50.0±2.9	45.0±2.9	40.0±2.9	26.7±1.7	16.7±1.7

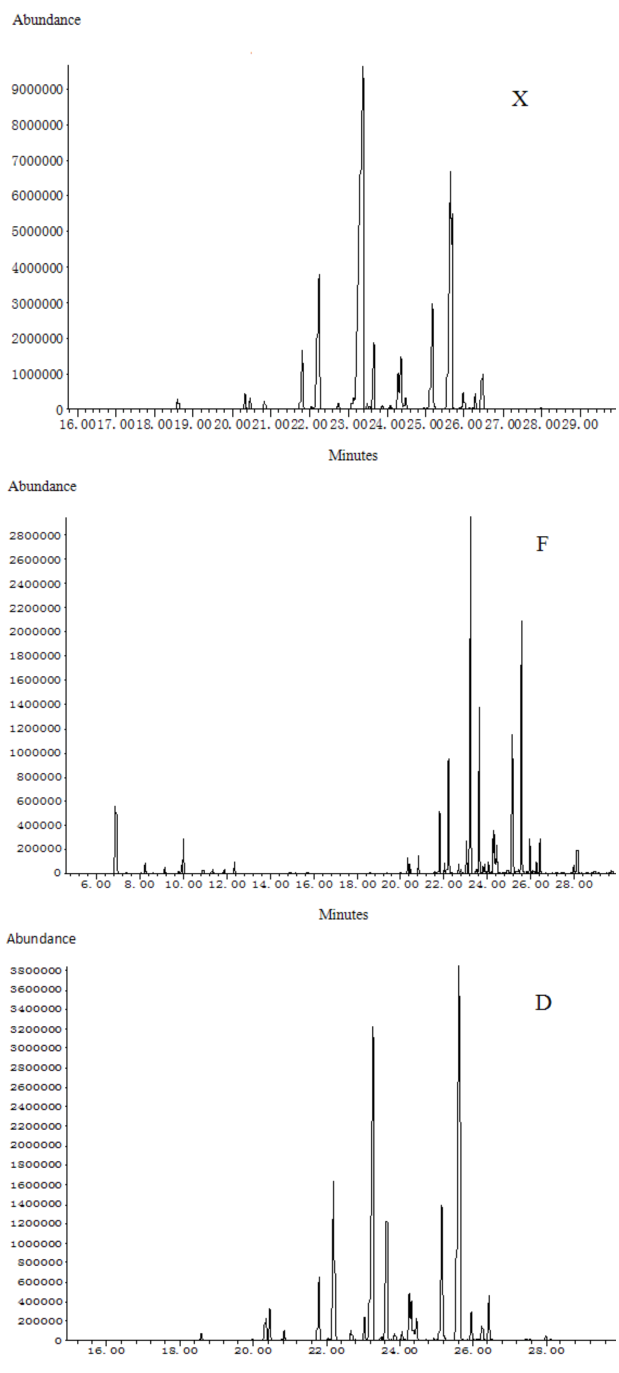

Fig. 5 GC-MS total ion chromatograms of mashed fresh(X), fallen(F), and dried(D) leaves of *M. exotica*.

Table 6. Chemical compositions from mashed fresh leaves of *M. exotica*.

Number	Composition	Relative retention time (min)	Percentage (%)
1	Artemisia triene	20.343	0.76
2	4-ethenyl-4-methyl-1-(propan-2-yl)-3-(prop-1-en-2-yl) cyclohexene	20.454	0.57
3	α-cubebene	20.842	0.36
4	α-copaene	21.810	3.09
5	(-)-β-bourbonene	22.050	0.18
6	Linalool	22.239	8.81
7	(Z,E)-α-farnesene	22.742	0.41
8	Bicyclo[3.1.1] hept-2-ene,2,6-dimetyl-6-(4-methyl-3-pentenyl)-	23.125	0.66
9	β-caryophyllene	23.381	40.20
10	(E)- β-farnesene	23.665	3.67
11	(-)-β-santalene	24.086	0.27
12	Humulene	24.370	3.01
13	Alloaromadendrene	24.477	0.60
14	β-copaene	24.951	0.09
15	Germacrene D	25.170	6.28
16	(+)-β-himachalene	25.640	17.76
17	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	25.689	7.00
18	β-bisabolene	25.978	0.88
19	d-cadinene	26.270	0.72
20	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	26.464	1.73

Table 7. Chemical compositions from mashed fallen leaves of *M. exotica*.

Number	Composition	Relative retention time (min)	Percentage (%)
1	(1R)-(+)- α-pinene	6.880	5.00
2	β-pinene	8.216	0.72
3	α-phellandrene	9.135	0.36
4	D-limonene	9.898	0.49
5	Eucalyptol	10.005	2.05
6	Linalool	12.350	0.61
7	Artemisia triene	20.330	0.89
8	4-ethenyl-4-methyl-1-(propan-2-yl)-3-(prop-1-en-2-yl) cyclohexene	20.442	0.48
9	α-cubebene	20.829	0.89
10	α-copaene	21.781	3.38
11	(-)-β-bourbonene	22.033	0.52
12	Germacrene D	22.185	6.90
13	Bicyclo[3.1.1] hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-	22.668	0.53
14	(Z,E)- α-farnesene	23.014	1.69
15	β-caryophyllene	23.241	25.30
16	trans-α-bergamotene	23.636	9.06
17	Alloaromadendrene	23.768	0.42
18	(-)-β-santalene	24.057	0.76
19	(E)-β-farnesene	24.255	2.61
20	Humulene	24.321	2.11
21	Aromadendrene	24.448	1.64
22	Curcumene	25.149	10.56
23	α-cedrene	25.574	14.01
24	l-β-bisabolene	25.949	1.83
25	d-cadinene	26.246	0.63
26	Cyclohexene,3-(1,5-dimethyl-4-hex enyl)-6-methylene-, [S-(R*,S*)]-	26.427	2.02
27	Espatulenol	27.981	0.50
28	Caryophyllene oxide	28.133	1.50

Table 8. Chemical compositions from mashed dried leaves of *M. exotica.*

Number	Composition	Relative retention time (min)	Percentage (%)
1	Camphene	20.339	1.11
2	4-ethenyl-4-methyl-1-(propan-2-yl)-3-(prop-1-en-2-yl) cyclohexene	20.458	1.59
3	α-cubebene	20.838	0.47
4	α-copaene	21.794	3.18
5	Germacrene D	22.210	9.13
6	(Z,E)-α-farnesene	22.684	0.50
7	Bicyclo[3.1.1] hept-2-ene,2,6-dimetyl-6-(4-methyl-3-pentenyl)-	23.039	1.22
8	β-caryophyllene	23.278	23.43
9	trans-α-bergamotene	23.649	6.38
10	cis-β-farnesene	23.871	0.38
11	epi-β-santalene	24.073	0.51
12	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	24.271	2.59
13	Humulene	24.337	1.97
14	Alloaromadendrene	24.461	1.12
15	β-cubebene	25.145	8.79
16	α-cedrene	25.619	25.05
17	Benzene, 1-(1,5-dimethylhexyl)-4-m ethyl-	25.648	7.56
18	β-bisabolene	25.969	1.39
19	d-cadinene	26.258	0.73
20	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	26.452	2.26
21	α-guaiene	27.985	0.25

Discussion

Our results have shown that the volatile compounds released from the mashed fresh, fallen, and dried leaves of *M. exotica* evidently reduce the grasping and walking abilities and aggregation rate of the RIFA workers. Furthermore, volatile compounds of the leaves of *M. exotica* have been showed to be insecticidal.

Plant insecticides are relatively safe, degradable, and readily available in many regions of the world; hence, these insecticides could replace some traditional chemicals. Li et al. (2014) reported that volatile compounds of *Tephrosia vogelii* exhibited high toxicity against the RIFA workers. The essential oils of *M. exotica* possessed fumigant toxicity against *Sitophilius zeamais*, and *Tribolium castaneum* adults with LC_{50} values of 8.29 and 6.84 mg/L, respectively. According Govindarajan et al. (2012), the essential oils from *Mentha spicata* (Linn.) exhibited larvicidal activity against three mosquito species, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The natural product of the leaves of *Boenninghausenia albiflora* was active against *Plecoptera reflexa*, *Clostera cupreata*, and *Crypsiptya coclesalis* at different concentrations varying from 1.0% to 5% w/v (Sharma et al. 2006). The acetone extract of *M. exotica* leaves showed an antifeedant activity against the early third-stage larvae of *Spodoptera litura* (Wang et al. 2009).

The results of the fumigation toxicity bioassay have shown that the fresh leaves of *M. exotica* is more active against RIFAs than fallen and dried leaves. The volatile compounds of mashed leaves of *M. exotica* are β-caryophyllene, α-cedrene, α-copaene, β-cubebene, and germacrene D. This finding is similar to that reported by Jiang et al. (2009). According to the results of GC–MS, β-caryophyllene is the major component of the leaves of *M. exotica*. The content of β-caryophyllene is highest in fresh leaves (40.20%) among in the fallen (25.30%) and in the dried leaves (23.43%). These observations show that the significant activity of *M. exotica* leaf may be due to the presence of major chemical constituents such as β-caryophyllene. Several studies reported that β-caryophyllene in other plants possessed insecticidal activity (Zhu & Tian, 2011; Krishnamoorthy et al., 2015; Venturi et al., 2015; Salleh et al., 2015). *M. exotica* as a popular hedge plant well adapted for topiary work is widely distributed in southern China and several tropical and subtropical regions of Asia. In these *M. exotica* growing areas, the plant need to trim every year and there are a lot of fresh leaves cut from it. Therefore the fresh leaves cut from *M. exotica* can be crushed thoroughly and used to control RIFAs.

The volatile compounds from the mashed fresh of *M. exotica* possess high insecticide activity against the RIFA workers. These facts indicate that the leaves of *M. exotica* have potential for controlling these pest ants. Fresh leaves of *M. exotica* could be collected and used as raw materials to control RIFA.

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